

*On the Possibility of Determining the Presence or Absence of Tubercular Infection by the Examination of a Patient's Blood and Tissue Fluids.*

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(Communicated by Sir John Burdon Sanderson, Bart., F.R.S. Received October 21,—Read November 23, 1905.)

In the present communication we propose (*a*) to set forth certain conclusions arrived at after the study of the tuberculo-opsonic power of the blood in a very considerable number of tubercular patients; (*b*) to show that we have in the measurement of the tuberculo-opsonic power of the blood and tissue fluids a method which may be exploited in the diagnosis of tubercular infection.

*Technique Employed.*—The technique employed by us in the measurement of the tuberculo-opsonic power of the blood was essentially that described by one of us in conjunction with Douglas.\* In each case the white corpuscles required for the tests were derived from blood from the finger received into a solution of 0.5 per cent. citrate of soda in 0.85 NaCl, and rewashed after centrifugalisation in a considerable volume of 0.85 NaCl, and then again centrifuged. Of the "blood-cream," obtained by skimming off the upper layer of the corpuscular sediment, one portion was in each case mixed in a capillary tube with one volume of serum and one volume of a suspension of tubercle bacilli which had been centrifuged in such a manner as to free it from bacillary clumps. After incubation at 37° for 15 to 20 minutes films were made on slides prepared with emery paper.† These films were, after fixture in saturated corrosive sublimate, stained with boiling carbol-fuchsin, decolourised with 2 per cent. sulphuric acid, and counter-stained with methylene blue after washing in 1 in 1000 sodium carbonate. The standard of comparison employed was obtained by mixing in each case the same "blood cream" and tubercle suspension with "pooled normal serum."‡

\* 'Roy. Soc. Proc.' vol. 72.

† Wright, 'Lancet,' July 9, 1904.

‡ While in this research pooled serum was employed, in order to provide against any chance variation of our bloods under the physical strain entailed by the work, it is to be noted that the observations of Urwick, conducted in this laboratory, and the more exten-

This "pooled serum" was obtained by mixing equal volumes of the sera of six to eight healthy students or laboratory workers. We have found that the opsonic power of such a "pooled serum" corresponds to the arithmetical mean of the opsonic indices of its component sera.

*Classification of Tubercular Cases into Strictly Localised Cases, and Cases which are associated with Constitutional Disturbance.*

Cases of tubercular infection distribute themselves in a natural manner under two headings. Into one category would fall the patients who are the subjects of a strictly localised infection unaccompanied by anything in the nature of constitutional disturbance. Cases where the infection is limited to one or more lymphatic glands; further, most cases of lupus, most cases of tubercular abscess in the subcutaneous tissue, tubercular affections of the joints, and, lastly, many stationary or only slowly progressing cases of tubercular phthisis, fall into this category.

Into another category would fall patients who are suffering from more generalised tubercular infections associated with constitutional disturbance. This group consists in large part of cases of pyrexial pulmonary tuberculosis. With these may be classed certain other cases of extensive or widely disseminated tuberculosis.

*Data with regard to the Tuberculo-opsonic Power in Cases of Strictly Localised Tuberculosis.*

The opsonic index is here low and uniformly low—in exceptional cases as low as one-sixth of the normal. Our findings in a series of cases of strictly localised tubercular infection are appended in tabular form below.

sive series of investigations carried out by Bulloch at the London Hospital ('Medico-Chirurg. Soc. Proc.,' 1905), and Lawson and Stewart at the Banchory Sanatorium (*loc. cit.*), have conclusively shown—(a) That the tuberculo-opsonic power of the blood does not in health range below 0·9 or above 1·1; and (b) that the bloods of A. E. W., S. R. D., and others which have hitherto in this laboratory furnished a standard of comparison, are, from the point of view of their tuberculo-opsonic power, typically normal bloods.

Table I.—Showing the Tuberculo-opsonic Index in a Series of Cases of strictly localised Tuberculosis.

Serial No.	Initials or, as the case may be, initial of patients.	Nature and seat of the infection.	Tuberculo-opsonic index.
1	J. R.	Tubercle of testis.....	0·65
2	A.	Caries, lower end of femur.....	0·7
3	C. S.	Tubercular ulceration, dorsum of hand	0·86
4	A. B.	" iritis.....	0·51
5	B. C.	" glands .....	0·4
6	E. M.	" ulceration of legs. 13 years' duration .....	0·17
7	H. W.	" glands (neck) .....	0·82
8	D. W.	" " .....	0·64
9	S.	" ulceration of legs .....	0·49
10	L. B.	" glands (abdominal).....	0·13
11	B.	" kidney .....	0·75
12	M. H.	" cystitis .....	0·85
13	D. B.	" glands. Extirpated and re-appeared .....	0·85
14	M. O. E.	" glands .....	0·6
15	W.	Psoas abscess .....	0·75
16	C. H.	" .....	0·65
17	W.	Tubercular glands (neck). 18 months' duration .....	0·47
18	C.	" wrist.....	0·85
19	U. R.	" glands (neck) .....	0·7
20	A. H.	" glands. Extirpated and re-appeared .....	0·54
21	P.	" peritonitis .....	0·6
22	R.	" abscesses and glands .....	0·6
23	E.	Lupus .....	0·6
24	R.	Tubercle of testes and bladder .....	0·72
25	P.	Tubercular peritonitis .....	0·7
26	H.	" caries of fibula .....	0·6
27	C.	Tubercle of kidney .....	0·88
28	W.	Tubercular disease of knee .....	0·6
29	T.	" glands (neck) recurrence ...	0·66
30	S.	" ulcer of foot .....	0·49
31	C.	" disease of knee .....	0·75

*Data with regard to the Tuberculo-opsonic Power of the Blood in Cases of Tuberculosis associated with Constitutional Disturbance.*

In the cases here in question the opsonic index of the blood is continually varying. The range of its fluctuation is from considerably under the normal to twice or more the normal height.

Striking examples of the variation of the opsonic index in connection with acute tubercular phthisis are furnished in the paper of our fellow-worker, R. H. Urwick, already referred to.\*

The following are instances of similar variation occurring in the subjects of other forms of tubercular infection :—

\* 'British Medical Journal,' July 22, 1905.

Example 1.—Child with Tubercular Caries of the Fibula, associated with Constitutional Disturbance.

Dates of blood examinations.	Tuberculo-opsonic index.
11.9.05	1.45
14.9.05	1.71
19.9.05	1.3
28.9.05	0.98
30.9.05	Operation, fibula scraped.
2.10.05	1.73*
3.10.05	1.13
10.10.05	1.3

Example 2.—Adult Patient with Tubercular Caries of the Spine and Constitutional Disturbance.

Date of blood examination.	Tuberculo-opsonic index.	Remarks.
19.5.05.....	0.65	} Temperature disturbance and pain associated with development of abscess.
20.5.05.....	1.4	
22.5.05.....	1.3	
23.5.05.....	1.0	} Temperature returns to normal in association with spontaneous discharge of abscess.
24.5.05.....	0.8	

Example 3.—Adult Patient with extensive Psoas Abscess and Generalisation of Tubercle. Case has since terminated fatally.

Date of blood examinations.	Tuberculo-opsonic index.
8.2.05	2
9.2.05	2.4
11.2.05	0.6

*Suggested Interpretation of the Different Findings in these two Categories of Cases.*

The explanation of the difference in the condition of the blood in these two contrasted categories of cases is probably the following: The condition of low opsonic power which is associated with strictly localised tuberculosis is almost certainly a condition which has preceded and has furnished the opportunity for infection. The fact that the opsonic index continues persistently

\*A rise in the opsonic power similar to this here registered has been repeatedly observed by us in connection with the stirring up by surgical interference of tubercular foci.

low after infection has supervened, while it can invariably be raised by appropriate inoculation,\* indicates that the machinery of immunisation with which the organism is furnished is not, under the conditions which obtain in strictly localised tubercular infections, spontaneously called into play.

The constant fluctuation in the opsonic power of the blood in cases of active pulmonary tuberculosis and other active forms of tubercular infection furnishes—as we can hardly doubt—evidence of a periodic conveyance of tubercular elements into the blood; and of a response to such stimulation on the part of the machinery for immunisation. The low opsonic indices registered in connection with active tuberculosis would in other words be “negative phases” such as supervene—as one of us has shown—upon the inoculation of all vaccines; the high opsonic indices would be “positive phases,” such as normally succeed upon the negative phases just mentioned; and the normal opsonic indices would correspond to periods of transition between negative and positive phases, or, as the case may be, to periods in which the blood is returning after a positive phase to the condition *quo ante*.

The life of a patient with any really active form of tuberculosis would in conformity with this view be a life of alternating negative and positive phases: the favourable or unfavourable event of the infection being in each case determined by the adjustment or want of adjustment of the auto-inoculations (with respect to dosage and interspacing) with the particular patient's capacity for immunising response.

Having now to a certain extent cleared the ground, we may pass on to consider the question of the diagnosis of tubercular infection by means of the measurement of the opsonic power of the blood.

*Exploitation of the data Summarised above as an aid in the Diagnosis of  
Tubercular Infection.*

Consideration will make clear that the data obtained by the measurement of the opsonic power in cases of doubtful diagnosis may, when adjudicated upon in the light of the data obtained in connection with undoubted cases of tuberculosis as given above, furnish material for admitting or rejecting the diagnosis of tubercular infection. We may formulate in connection with this matter the following propositions:—

(1) Conclusions which can be arrived at when we have at disposal the results of a series of measurements.

(a) *Where a series of measurements of the opsonic power of the blood reveals a*

\* Exactly the same statements hold true with regard to the staphylo-opsonic power in localised staphylococcus infections (furunculosis, sycosis, etc.).

*persistently low opsonic power with respect to the tubercle bacillus, it may be inferred, in the case where there is evidence of a localised bacterial infection which suggests tuberculosis, that the infection in question is tubercular in character.*

*(b) Where repeated examination reveals a persistently normal opsonic power with respect to the tubercle bacillus, the diagnosis of tubercle may with probability be excluded.*

Illustrative case: A. B.—Case diagnosed as tubercular cystitis on the evidence of pus in the urine, of the cystoscopic appearances and general disturbance of health. The measurement of the tuberculo-opsonic power of the blood yielded the following results:—

Date of blood examination.	Tuberculo-opsonic index.
2.3.05	0.98
14.4.05	0.99
28.4.05	1
18.5.05	1
19.5.05	1.1
2.10.05	0.97

The inference that the cystitis and disturbance of health was not of tubercular origin was confirmed (*a*) by the fact that an extensive series of bacteriological examinations prolonged over many months revealed in every case the presence of proteus in large numbers, while the tubercle bacillus was never found, even when examined for by the inoscopic method of Jousset; (*b*) by the fact that the patient's blood possessed, anterior to treatment with regard to the proteus, an agglutinating power which was three times higher than the normal; and (*c*) by the fact that very striking amelioration of the cystitis, and a complete return to health has been obtained as the result of the inoculation of a proteus vaccine.

*(c) Where there is revealed by a series of blood examinations a constantly fluctuating opsonic index the presence of active tuberculosis may be inferred.*

C. D.—A case of severe chronic urticaria of unknown ætiology. The measurement of the tuberculo-opsonic power of the patient's blood yielded the following results:—

Date of blood examination.	Tuberculo-opsonic index.
20.5.05	1.3
26.5.05	1.3
16.6.05	0.86
20.6.05	1.27

The inference drawn from these data that the patient was suffering from some active form of tuberculosis was confirmed (a) by the discovery by an independent observer of a lesion in the apex of one lung; (b) by the subsequent development of an abscess of an obviously tubercular character; and (c) by the marked improvement in health which has followed upon inoculation with tubercle vaccine.

(2) Conclusions which may be arrived at where we have at disposal the result of an isolated blood examination.

(a) *Where an isolated blood examination reveals that the tuberculo-opsonic power of the blood is low, we may—according as we have evidence of a localised bacterial infection or of constitutional disturbance—infer with probability that we are dealing with tuberculosis—in the former case with a localised tubercular infection, in the latter with an active systemic infection.*

(b) *Where an isolated blood examination reveals that the tuberculo-opsonic power of the blood is high, we may infer that we have to deal with a systemic tuberculous infection which is active, or has recently been active.*

(c) *Where the tuberculo-opsonic power is found normal, or nearly normal, while there are symptoms which suggest tuberculosis, we are not warranted, apart from the further test described below, in arriving at a positive or a negative diagnosis.*

*Discrimination of Tubercular Blood from Normal Blood by the aid of the Phagocytic Test Conducted with Serum which has been subjected to a Temperature of 60° C.*

The further criterion to which reference was made in the preceding paragraph is the following:—

*When a serum is found to retain in any considerable measure, after it has been heated to 60° for 10 minutes, its power of inciting phagocytosis we may conclude that “incitor elements”\* have been elaborated in the organism either in response to auto-inoculations occurring spontaneously in the course of tubercular infection, or, as the case may be, under the artificial stimulus supplied by the inoculation of tubercle vaccine.*

A typical selection from the very extensive body of observations which furnishes the basis of the above statement is presented in Tables II and III.

It will be seen from these tables that in practically every case where a reaction to tubercular infection may be assumed to have taken place,

\* The term “incitor elements” (Latin, *incito*, I urge forward, I hasten, I bring into rapid movement) is here employed in lieu of a more specific term, in order not to prejudge the mode of action of the element in the heated serum which promotes phagocytosis. The nature of the incitor element is considered in the next following communication.

Table II.—Showing that the Normal Serum, after it has been exposed to a Temperature of 60° C. for 10 minutes, no longer incites Phagocytosis.\*

Serial number of the observation.	Derivation of the serum.	Unheated serum.		Heated serum.	
		Phagocytic count. (Number of bacteria ingested divided by number of leucocytes examined.)	Tuberculo-opsonic index.	Phagocytic count. (Number of bacteria ingested divided by number of leucocytes examined.)	Tuberculo-opsonic index.
1	Healthy man .....	(104/40) = 2·6	Taken as 1	(13/40) = 0·32	0·125
2	" .....	(96/40) = 2·4	" 1	(8/40) = 0·2	0·08
3	Pooled serum of six healthy men	(247/36) = 6·8	" 1	(30/50) = 0·6	0·09
4	Healthy boy.....	(250/39) = 6·4	" 1	(15/40) = 0·4	0·06
5	" .....	(214/30) = 7·0	" 1	(19/40) = 0·47	0·06
6	Pooled serum of eight normal men	(60/50) = 1·2	" 1	(2/20) = 0·1	0·08
7	Healthy man .....	(55/40) = 1·4	" 1	(0/40) = 0·0	0·00
8	Pooled serum of six healthy men	(132/30) = 4·4	" 1	(3/30) = 0·1	0·1

evidence of that reaction can be obtained by conducting the phagocytic test with serum which has been heated to 60° C. for 10 minutes.

The observations numbered 15 and 16 respectively have, it may be noted, been introduced into the table with the special design of showing the very simple nature of the investigation which is required for the diagnosis of tubercle in the case where that infection has called forth a reaction of immunisation.

The following observations, which we owe to our fellow-worker Dr. G. W. Ross, bring out in an instructive manner the trustworthiness of the phagocytic test with heated serum as applied in this its simplest form :—

*Case 1.—Girl, æt. six years, Tentatively Diagnosed Pulmonary Phthisis.*

Phagocytosis obtained with the serum, heated for 10 minutes to 60° C. and employed in a phagocytic mixture containing over 1 per cent. NaCl.

The verdict of tubercular infection of the lung which was based on this was confirmed on *post-mortem* examination.

\* In order to avoid the fallacies associated with spontaneous phagocytosis (*vide* the next following communication) the observations which are recorded in this and in the subsequent table were in each case made by mixing the volume of the serum with one volume of corpuscles, washed in 0·85-per-cent. NaCl solution and one volume of tubercle bacilli suspended in a 1·5-per-cent. NaCl solution. In this manner a salt content of over 1-per-cent. NaCl was achieved in the phagocytic mixture.



Table III.—Showing that an element which incites Phagocytosis is contained in the heated serum of patients who are the subjects of an active systemic tubercular infection, or who have been subjected to inoculations of a tubercle vaccine. The sera, like those which are in question in Table II, were in each case heated to 60° C. for 10 minutes.

Serial number of observation.	Nature of infection.	Unheated serum.		Heated serum.	
		Phagocytic count. (Number of bacteria ingested divided by number of leucocytes examined.)	Tuberculo- opsonic index (determined by comparison of phagocytic count with that obtained with pooled blood of healthy men).	Phagocytic count. (Number of bacteria ingested divided by number of leucocytes examined.)	Tuberculo- opsonic index (determined by comparison of phagocytic count with that obtained with unheated pooled blood of normal men).
1	Tubercular caries of hip	—	1·5	—	0·4
2	Tubercular phthisis ...	(125/20) = 6·2	1·4	(113/30) = 3·7	0·8
3	„ ...	(152/30) = 5·0	1·2	(96/30) = 3·2	0·72
4	„ ...	(98/30) = 3·2	1·0	(20/65) = 0·3	0·1
5	Tubercular peritonitis	(144/30) = 4·8	1·4	(103/30) = 3·4	1·0
6	„ ...	(142/30) = 4·7	1·4	(16/50) = 0·3	0·09
7	Phthisis and tubercular glands	(113/40) = 2·8	1·1	(79/50) = 1·6	0·6
8	Tubercular caries of hip	(110/30) = 3·6	1·0	(85/30) = 2·8	0·8
9	Tubercular abscess of kidney	—	1·7	(26/30) = 0·8	0·4
10	Lupus under treatment by inoculation of tubercle vaccine	(34/10) = 3·4	0·7	(49/30) = 1·6	0·33
11	Tubercular ulcer of leg under treatment by inoculation of tubercle vaccine	(249/40) = 6·2	1·2	(149/40) = 3·7	0·7
12	Tubercle of kidney under treatment by inoculation of tubercle vaccine	(68/40) = 1·7	1·5	(77/40) = 1·9	1·7
13	Tubercular glands and abscess under treatment by inoculation of tubercle vaccine	(59/40) = 1·5	1·4	(36/40) = 0·9	0·8
14	Tubercular cystitis under treatment by inoculation of tubercle vaccine	(97/50) = 2·0	—	(43/30) = 1·4	—
15	Phthisis .....	—	—	(26/30) = 0·8	—
16	„ .....	—	—	(9/5) = 1·8	—

*Case 2.—Man, æt. 41, Tentative Diagnosis, Pleurisy due to Malignant Disease, or Tubercular Pleurisy.*

No phagocytosis obtained with the serum, heated for 10 minutes to 60° C. and employed in a phagocytic mixture containing over 1 per cent. NaCl.

The verdict of pleurisy due to malignant disease, which was based on this, was confirmed on *post-mortem* examination.

*Case 3.—Case Tentatively Diagnosed Miliary Tuberculosis or Malignant Endocarditis.*

No phagocytosis obtained with the serum, heated for 10 minutes to 60° C. and employed in a phagocytic mixture containing over 1 per cent. NaCl.

The verdict of malignant endocarditis which was based on this was confirmed on *post-mortem* examination.

*Observation 4.—Case Diagnosed Miliary Tuberculosis.*

No phagocytosis obtained with the serum, heated for 10 minutes to 60° C. and employed in a phagocytic mixture containing over 1 per cent. of NaCl.

The *post-mortem* examination revealed a complete absence of tubercular lesions and a healing typhoid ulcer\* in the ileum.

*On two other Methods by which a Diagnosis of Tubercular Infection can be arrived at or Excluded.*

In addition to the methods which have been already considered, there are two further methods which can be exploited in connection with the diagnosis of tubercular infection. The first of these is applicable where we desire to supplement the often ambiguous data furnished by the clinical symptoms in the case of inoculations of tuberculin undertaken for diagnostic purposes. The second is applicable where we can obtain, in addition to the patient's blood, also lymph, or, as the case may be, pus from the seat of infection.

*Diagnosis of Tubercular Infection by the Aid of Measurements of the Opsonic Power carried out in Connection with the Inoculation of Tuberculin for Diagnostic Purposes.*

Already, three years ago,† in connection with a paper on staphylococcus inoculations as applied to the treatment of acne, furunculosis, and sycosis, attention was directed by one of us to the close analogy between the tubercular reaction of Koch and the local inflammation and general constitutional disturbance which may supervene when a patient whose

\* A negative Durham-Gruber reaction had been obtained in this case.

† 'Lancet,' March 29, 1902.

tissues are extensively invaded by the staphylococcus is inoculated with the corresponding vaccine in such a manner as to develop a pronounced negative phase.

The association of a negative phase with a reaction similar to that conveniently spoken of as the *tuberculin reaction*, suggested to us the propriety of enquiring whether the true tuberculin reaction, as seen after the injection of Koch's old tuberculin into a tubercular patient, was also associated with a negative phase.

The opportunities for investigating the question which have presented themselves have not yet been sufficiently numerous to allow of our formulating a final answer to this question. The observations which are set forth below seem to us to suggest that the development of a negative phase, with a dose of tuberculin smaller than that which would produce this result in a healthy patient, may prove to be an index of tubercular infection. Such a conclusion would be in harmony with our experience in connection with the therapeutic inoculation of tubercle vaccine (new tuberculin). We find in this connection that the negative phase supervenes upon very much smaller doses and persists much longer in the case where the patient is the subject of extensive infection than in the contrary case.

*Observation 1.*—Case diagnosed, Tubercular choroiditis.

Date.	Tuberculo-opsonic index.	Clinical data.
26.4.05.....	0·9	Some constitutional reaction, <i>t.</i> 100° F.
5 milligrammes old tuberculin inoculated.		
29.4.05.....	0·29	
28.4.05.....	0·95	

*Observation 2.*—Case diagnosed, Lupus erythematosus.

Date.	Tuberculo-opsonic index.	Clinical data.
12.1.05.....	0·73	No rise of temperature or constitutional or local reaction.
Inoculation of 1 milligramme old tuberculin.		
13.1.05.....	0·85	
17.1.05.....	1·6	
26.1.05.....	0·5	

*Observation 3.*—Case diagnosed, Lupus erythematosus.

Date.	Tuberculo-opsonic index.	Clinical data.
10.4.05.....	0·66	Quite insignificant constitutional disturbance.
Inoculation of 5 milligrammes of old tuberculin.		
11.4.05.....	0·7	
12.4.05.....	1·2	
14.4.05.....	0·85	

*Observation 4.*—Case diagnosed as Lupus vulgaris.

Date.	Tuberculo-opsonic index.	Clinical data.
10.4.05.....	0·55	Quite insignificant constitutional reaction.
Inoculation of 5 milligrammes of old tuberculin.		
11.4.05.....	1·1	
12.4.05.....	1·0	
14.4.05.....	1·0	

*Observation 5.*—Lupus, patient had been treated for many months by therapeutic inoculations of tubercle vaccine.

Date.	Tuberculo-opsonic index.	Clinical data.
24.1.05.....	1·4	Severe constitutional and local reaction, <i>t.</i> of 103° F.
Inoculation of 30 milligrammes of tuberculin.		
25.1.05.....	0·34	
26.1.05.....	2·1	
27.1.05.....	1·7	

*Diagnosis of Tubercular Infection by the Comparison of the Opsonic Power of the Patient's Blood with the Tuberculo-opsonic Power of the Fluids Derived from the Focus of Infection.*

Attention has already been drawn by one of us, both in a research undertaken in conjunction with Lamb\* and in a research undertaken in conjunction with Douglas,† to the fact that we have in the actual focus of

\* 'Lancet,' December 23, 1899.

† 'Roy. Soc. Proc.,' vol. 74, p. 157.

infection a lowered "bacteriotropic pressure" which accounts for the cultivation of the pathogenetic microbe in the interior of an organism which has at disposal in the circulating blood a considerable reserve of anti-bacterial substances. We propose here in conclusion to furnish further illustration of the general law as enunciated above, culling our examples not alone from the observations we have made in connection with tubercular infection, but also from observations made in connection with other bacterial infections.

*Observation 1.—Case of abscess in the neighbourhood of the appendix.*

Blood from the patient's finger and pus obtained from the abscess at the operation were examined, with a view to determining the nature of the infection.

	Phagocytic counts.	
	With a suspension of tubercle bacilli.	With a suspension of staphylococci.
Serum .....	2·3	4·5
Fluid obtained from the pus by centrifugalisation	0·1	1·9

The fact that the tuberculo-opsonic power of the patient's blood was here 23 times as great as that of the fluid obtained from the pus was taken as evidence that tuberculo-opsonic substances had been used up in the pus and that the patient was suffering from a tubercular infection. It was inferred on similar grounds that he was also infected by staphylococcus.

*Observation 2.—Case of osteo-myelitis of the femur.* Blood from the patient's finger and pus obtained from the abscess at the operation were examined, with a view to determining the nature of the infection.

	Tuberculo-opsonic index.	Staphylo-opsonic index.
Serum .....	1·0	2·5
Fluid obtained from the pus by centrifugalisation	1·1	0·9

The fact that the opsonic index of the patient's circulating blood was here normal to tubercle, while it was two and a-half times greater than normal

with respect to the staphylococcus, was taken as evidence that the patient was not infected with tubercle, and that he was infected by staphylococcus, and had responded to that infection by a production of immunising substances.

The fact that the tuberculo-opsonic index of the fluids obtained from the pus was the same as that of the blood, while the staphylo-opsonic power was only two-fifths of that of the circulating blood, was taken as of confirmatory evidence of the conclusion already arrived at. The fact that a copious culture of staphylococcus aureus was obtained from the pus, planted out with aseptic precautions at the operation, further confirmed the diagnosis.

*Observation 3.—Case of psoas abscess.* Blood from the patient's finger and pus from the abscess were examined.

	Phagocytic counts.	
	With a suspension of tubercle bacilli.	With a suspension of staphylococci.
Serum .....	2·4	5·0
Fluid obtained from the pus by centrifugalisation	1·23	1·2

The fact that the fluid obtained from the pus was impoverished in both tuberculo- and staphylo-opsonic substances as compared with the blood was taken as evidence of a combined infection by tubercle bacilli and staphylococci. This inference was confirmed by the fact that the opsonic power of the blood with respect to both the micro-organisms here in question was undergoing perpetual fluctuations.\* The inference so far as it related to the staphylococcus was further confirmed by the fact that cultures of the micro-organism were obtained from the pus.

*Observation 4.—Case of ascites with grave constitutional disturbance in a man of 30.* Blood from the finger and ascitic fluid were examined on two occasions.

#### First Occasion.

##### Tuberculo-opsonic index.

Serum..... 1·05

Ascitic fluid ..... 0·99

We reported upon this that the patient was not suffering from tubercular peritonitis.

\* For the variations registered in connection with the tuberculo-opsonic power, *vide supra*, p. 196 of this paper, where Example 3 refers to the patient here in question.

The clinical symptoms, the age of the patient, and the appearances as seen at the operation appearing in contradiction with this verdict, and the ascites having reappeared, a second operation was performed, and a further sample of ascitic fluid was obtained for examination. At the same time the clinical appearances were again observed, with the result that there was now some wavering as to whether the original diagnosis could be upheld. The result of the phagocytic examination of the ascitic fluid, and of a sample of blood from the fingers were now as under :—

## Tuberculo-opsonic index.

Serum .....	1
Ascitic fluid.....	1

In view of this result the verdict previously given was sustained.

A *post-mortem* examination, which followed in the course of a few weeks, again threw doubt on the verdict, the naked-eye appearances being entirely consistent with the theory of miliary tuberculosis affecting the peritoneum and serous covering of the intestines. Microscopic examination of the sections made through the miliary nodules revealed, however, a typical picture of miliary carcinoma. No primary carcinomatous focus had been discovered, though it was sought for on *post-mortem* examination.

*Observation 5.—Case of pleural effusion.* Blood from the finger and fluid obtained by paracentesis of chest were examined :—

## Tuberculo-opsonic index.

Serum.....	0·92
Pleural fluid .....	1·0

This was taken as evidence of the absence of tubercular infection.

*Observation 6.—Case diagnosed as peritoneal tubercular peritonitis complicated with pleurisy.* Blood from the finger was examined on two occasions. On the second occasion, which was 48 hours after the first examination, peritoneal and pleural fluid were also examined.

The results obtained by the phagocytic examination undertaken on this second occasion were as follows :—

## Tuberculo-opsonic index.

Serum.....	0·7
Peritoneal fluid .....	0·28
Pleural fluid .....	1

The results of the comparison of the peritoneal fluid with the serum obtained from the blood withdrawn from the finger were taken as evidence of tubercular infection of the peritoneum. Confirmatory evidence of tubercular infection was furnished further by the low tuberculo-opsonic

power of the blood, and by the observed fluctuation in this index. When it was measured two days previously, this index had worked out as 1·4.

The fact that the opsonic power of the pleural fluid worked out as higher than the opsonic power of the serum was taken as evidence that the pleural effusion had occurred at a period when the opsonic power of the blood was 1 or above 1.

The diagnosis of tubercular infection of the peritoneum and pleura (and underlying lung) was confirmed at the *post-mortem* examination.

*Observation 7.*—*Case of long-continued suppuration of the antrum* associated with the presence in the pus of the pneumococcus and the *Bacillus fusiformis* and *Spirillum buccæ* of Vincent. The patient had been treated by therapeutic inoculations of a pneumococcus vaccine. The patient's serum and the antral pus were examined with a view to determining whether the pneumococcus played any active part in connection with the continuance of the suppuration:—

	Pneumo-opsonic index.
Serum .....	4·3
Fluid obtained from pus by centrifugalisation .....	0·3

The results were taken as evidence (*a*) that the pneumococcus played an active rôle in connection with the suppuration, and (*b*) that the protective substances which had been generated in the blood under the influence of inoculation did not come satisfactorily into application upon the micro-organisms in the antrum.

*Observation 8.*—*Case of whitlow associated with the formation of a blister under the nail.* Serum derived from blood from a sound finger and blister fluid were examined.

	Staphylo-opsonic index.
Serum .....	0·8
Blister fluid .....	0·3

The blister fluid yielded a pure culture of staphylococcus.

*Observation 9.*—*Rabbit in the early stages of anthrax infection.*—Blood obtained from the ear and lymph from the seat of inoculation were examined.

	Anthraco-opsonic index.*
Serum .....	1·7
Lymph .....	0·62

\* Tested with a suspension of anthrax spores and compared with the serum of a normal rabbit tested in the same manner.

It may be noted that all the difficulties and inaccuracies which are associated with the employment of ordinary anthrax cultures in phagocytic experiments can be satisfactorily evaded by the employment of suspensions of anthrax spores. These, when stained with carbol fuchsin and decolourised by 0·25 per cent. sulphuric acid, represent absolutely ideal elements for enumeration.



## APPENDIX.

*A further Series of Observations showing that Phagocytosis is obtained with the Heated Serum of Patients who are the subjects of a Systemic as distinguished from a strictly Localised Tubercular Infection, or who, being the subjects of a strictly Localised Tubercular Infection, have been subjected to Inoculations with Tubercle Vaccine. The serum was in each case heated to 60° C. for 10 minutes.*

Table supplementary to Table II.—Showing that the Normal Serum, after it has been exposed to a Temperature of 60° C. for 10 minutes, no longer incites Phagocytosis.

Serial number of observation.	Nature of infection.	Unheated serum.		Heated serum.	
		Phagocytic count. (Number of bacteria ingested divided by number of leucocytes examined.)	Opsonic index (determined by comparison of phagocytic count with that obtained with pooled blood of healthy men).	Phagocytic count. (Number of bacteria ingested divided by number of leucocytes examined.)	Opsonic index (determined by comparison of phagocytic count with that obtained with pooled unheated blood of normal men).
1	Fibroid phthisis, tubercle bacilli in sputum	(100/30) = 3·3	1·0	(142/37) = 4·0	1·2
2	Early phthisis, tubercle bacilli in sputum	(132/30) = 4·4	1·3	(122/47) = 2·6	0·77
3	Acute phthisis, tubercle bacilli in sputum	(130/30) = 4·3	1·3	(96/40) = 2·4	0·74
4	Acute phthisis .....	(127/40) = 3·2	1·0	(45/34) = 1·3	0·4
5	Fibroid phthisis (?) ...	(182/30) = 6·0	1·8	(51/43) = 1·2	0·3
6	Phthisis, tubercle bacilli in sputum	(117/30) = 3·9	1·1	(65/30) = 2·2	0·62
7	Mitral stenosis .....	(106/30) = 3·5	1·0	(19/31) = 0·6	0·17
8	Early phthisis .....	(161/30) = 5·4	1·6	(54/27) = 2·0	0·6
9	Phthisis .....	(257/40) = 6·4	1·3	(51/40) = 1·3	0·27
10	Lupus under treatment by inoculation of tubercle vaccine	(131/36) = 3·3	1·6	(74/40) = 1·8	0·8
11	Lupus under treatment by inoculation of tubercle vaccine	(73/30) = 2·4	1·2	(31/30) = 1·0	0·5
12	Tubercular ulcer of leg under treatment by inoculation of tubercle vaccine	(63/30) = 2·1	1·2	(60/30) = 2·0	1·1

The first eight of the observations here in question were made upon bloods collected for us in the Victoria Park Hospital by our fellow worker, Dr. G. W. Ross. The clinical diagnosis which had been arrived at was not made known to us till afterwards, when the particulars set forth in Column 2 were filled in by Dr. Ross.

In contrast with the observations incorporated in Table II in the body of the paper these observations were conducted in phagocytic mixtures containing 0·85 per cent. instead of 1·1 per cent. of NaCl. It is shown in the next following communication that spontaneous phagocytosis is absolutely abolished only in the case when the salt content of the phagocytic mixture exceeds 1 per cent.

The source of fallacy to which attention is here called falls, no doubt, for all practical purposes, entirely out of account.

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*On Spontaneous Phagocytosis, and on the Phagocytosis which is Obtained with the Heated Serum of Patients who have Responded to Tubercular Infection, or, as the case may be, to the Inoculation of a Tubercle Vaccine.*

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(Communicated by Sir John Burdon-Sanderson, Bart., F.R.S. Received October 21,—Read November 23, 1905.)

It has been indicated in the foregoing paper than an *incitor element*\* is to be found in the blood of those who have made an immunising response to tubercular infection, or, as the case may be, to an inoculation of a tubercle vaccine. This fact does not stand by itself.

*Recital of Previous Observations on the same Subject.*

The observations of *Metchnikoff*, following in sequence upon the classical researches of R. Pfeiffer on the intraperitoneal destruction of bacteria by the aid of immune sera, first drew attention to the fact that very active phagocytosis comes under observation when bacterial cultures, or as the case may be spermatozoa, are introduced into the peritoneal cavity of normal animals in association with heated serum derived from immunised animals.

\* The term "incitor-element" (Latin—*incito*: I hasten, I urge forward, I bring into rapid movement) is here employed to denote the element in the heated serum which promotes phagocytosis. By employing this term, pending the elucidation of the nature and mode of action of the element in question, we secure the advantage of leaving these issues unprejudged.